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S100A4 (A Metastasis-Associated Protein)-Dependent
Increase in Motility of Breast Cancer Cells

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INTRODUCTION

Breast cancer mortality most often directly results from metastatic spread to distal, vital organs. When breast carcinomas metastasize, they must alter their phenotype to facilitate movement (1). The molecular differences that dictate whether a lesion metastasizes are not clearly understood. Therefore there is a clear need to identify and characterize key molecular elements that dictate the phenotypic changes between a relatively benign local hyperplasia, in situ carcinoma, and a malignant carcinoma. The S100A4 protein has recently become a strong candidate as a molecular marker for metastatic potential with high prognostic significance.

BODY

S100A4 (also known as pEL-98, 18A2, p9Ka, CAPL, calvasculin, and Fsp1) is a member of the S100 family of calcium binding proteins. Members of this family have been implicated in cytoskeletal-membrane interactions, calcium signal transduction, and cellular growth and differentiation (2). An increase in S100A4 protein expression has been correlated with a poor prognosis for patients with different types of cancer including: colorectal; gall bladder; esophageal; breast; and nonsmall lung cancer (3). It is strategically imperative that we develop a better understanding of how the expression of specific metastasis-promoting proteins, like S100A4, propels a tumor toward malignancy.

The function of S100A4 in metastasis is still unclear. S100A4 is alone not tumorigenic, but rather an inducer of metastasis in an already tumorigenic background (4). Reports have shown that transgenic mice overexpressing S100A4 do not develop tumors. However the progeny from mice overexpressing S100A4 crossed with mice overexpressing the *HER2/Neu* oncogene develop tumors that metastasize more frequently and more rapidly than tumors in the parental *neu* mice (5). By characterizing the cooperation between S100A4 and the ErbB2/ *HER2/Neu* oncogene, we may gain further insight into the role of S100A4 in metastasis.

Hence, I am using ErbB2 as a model oncogene to study the interaction between oncogenes and regulators of cell architecture such as S100A4 during the transformation of mammary epithelium. Oncogenes in the ErbB family of receptor tyrosine kinases play important roles in breast cancer. Among its four members, ErbB2 (Her2/Neu) overexpression is observed in 25-35% of breast cancers and correlates with a poor clinical prognosis (6)

By identifying and characterizing the interaction of S100A4 with other known oncogenes, we can supplement our understanding of how this particular clinical marker mechanistically engages cells to adopt a more motile, and thereby, more metastatic phenotype. Ultimately, this greater understanding will ideally lead to the use of S100A4 as not only a diagnostic marker but also as a target for therapeutic design.

KEY RESEARCH ACCOMPLISHMENTS

As outlined in Task 1, I am currently working on the mechanisms by which the receptor tyrosine kinase ErbB2 transforms human breast epithelial cells. Using a small molecule ligand based dimerization study to inducibly activate ErbB2 receptor in polarized, proliferation arrested epithelial cells, it was shown that activation of ErbB2 results in deregulation of proliferation control and disruption of cellular architecture (7). I am using this dimerization system to study the role of ErbB2 in S100A4 induced metastasis.

We are using the human mammary epithelial cell line, MCF10A, which was retrovirally infected with an inducible ErbB2 chimera. In accordance with Task 1, these cells have been fully characterized on plastic dishes as well as a three-dimensional cell culture method (Figure 1).

Since activation of ErbB2 in MCF-10A acini induces formation of non-invasive structures, I am working to determine whether S100A4 cooperates with ErbB2 in inducing invasive progression.

Before transfer of the grant, the previous PI (Edward Kim) was able to generate MCF10A cell lines overexpressing S100A4. The cDNA was subcloned into a pBabe.Puro retroviral vector to generate retrovirus expressing S100A4. MCF-10A cells were infected and selected with 1.0 ug/ml puromycin for three days. Resistant populations were generated. However, the cells were not characterized. My goal, in accordance with Task 1, is to characterize these cell lines to ensure expression of the protein and to examine their ability to grow in both 2D and 3D as compared to parental cells. I am working to introduce the well-established inducible ErbB2 chimera into the MCF10A-S100A4 cell lines, so as to characterize any cooperation between S100A4 and ErbB2.

My goal in Task 2 is to identify S100A4 associated proteins that are regulated by ErbB2. To this extent I have optimized conditions of activation ErbB2 in the MCF10A cell lines. The ErbB2 receptor is inducibly tyrosine phosphorylated and successfully activates known downstream pathways, such as the MAP Kinase pathway (Figure 2). I have collected lysates after different time points of ErbB2 stimulation and am looking at the presence or absence of S100A4 using a specific antibody, as well as association of ErbB2 and S100A4. Due to difficulty creating MCF10A-S100A4 cell lines that overexpress ErbB2, I have yet to determine changes in S100A4 associated complex by immunoprecipitation. The experiments outlined in Task 2 are ongoing.

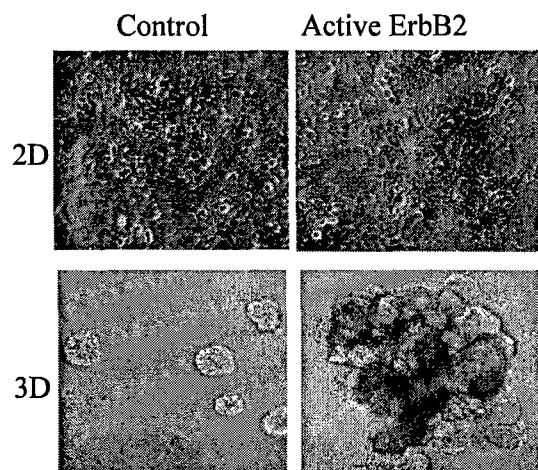


Figure 1: (Top) MCF10A cells expressing the ErbB2 chimera grown under plastic culture conditions. Cells were grown to confluency and dimerizer was added for 3 hours to activate ErbB2. (Bottom) MCF10A cells grown under Matrigel 3D cell culture conditions, represent day 12 structures with and without active ErbB2 (7).

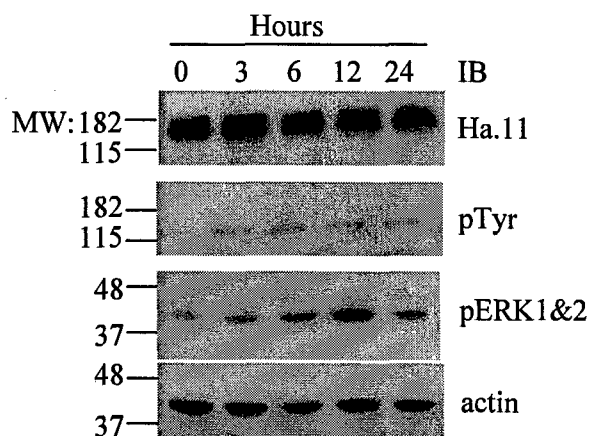


Figure 2: MCF10A cells expressing ErbB2 chimera were stimulated with dimerizer to activate ErbB2 for hours shown, lysed in RIPA buffer, and immunoblotted. Ha.11 (tag) MW= 180kDa
Phosphotyrosine MW= 180kDa
PhosphoERK 1 and 2 MW= 42, 44kDa
Actin (loading control) MW= 44kDa

REPORTABLE OUTCOMES

None

CONCLUSIONS

Since transfer of the award to me in October of 2004, I have completed research that provides a solid foundation for accomplishing my set tasks. I have obtained all necessary reagents, and have mastered all applicable assays. The ErbB2 expressing MCF10-A cell line has been fully established and characterized, and ErbB2 alone shows no metastatic phenotype, both in 2D and 3D culture.

MCF10A cells that overexpress S100A4 have been created, and are undergoing characterization. MCF10A-S100A4 cell lines expressing the ErbB2 chimera are being generated. I plan to have this done within the next few months, so that I can move forward with the research.

My goal is to determine whether the loss of epithelial architecture triggers an increase in epithelial cell proliferation and invasion characteristic of breast carcinoma and eventual metastasis. Because S100A4 regulates cell architecture as well as cell motility, understanding the mechanism by which S100A4 cooperates with ErbB2 will provide the much-needed insight into the observation that overexpression of S100A4 correlates with metastatic disease. By this, I aim to uncover novel mechanisms involved in the initiation of epithelial cell transformation – a major step towards developing new drugs for the treatment and ultimately prevention of breast cancer.

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